CHRONIC TOXICITY SUMMARY

CARBON TETRACHLORIDE

(carbon chloride; carbon tet; freon 10; halon-104; methane tetrachloride; necatrine; tetrachlorocarbon; tetrachloromethane; tetraform; tetrasol; univerm)

CAS Registry Number: 56-23-5

I.   Chronic Toxicity Summary

Inhalation reference exposure level	**40 ng/m^3** (6 ppb)
Critical effect(s)	Increased liver weight and hepatic fatty infiltration in guinea pigs
Hazard index target(s)	Alimentary system; development (teratogenicity); nervous system

II.   Physical and Chemical Properties (HSDB, 1995; CRC, 1994)

Description	Colorless liquid
Molecular formula	CCl_4
Molecular weight	153.8 g/mol
Density	1.59 g/cm^3 @ 20°C
Boiling point	76.7°C
Melting point	−23°C
Vapor pressure	91.3 torr @ 20°C
Solubility	Soluble in acetone, ethanol, benzene, carbon disulfide, slightly soluble in water
Conversion factor	1 ppm = 6.3 mg/m^3 @ 25°C

III.   Major Uses or Sources

Carbon tetrachloride was formerly used for metal degreasing and as a dry-cleaning fluid, fabric-spotting fluid, fire-extinguisher fluid, grain fumigant and reaction medium (DeShon, 1979). Carbon tetrachloride is used as a solvent for the recovery of tin in tin-plating waste and in the manufacture of semiconductors. It is used in petrol additives, refrigerants, metal degreasing, and as a catalyst in the production of polymers. Carbon tetrachloride is also used as a chemical intermediate in the production of fluorocarbons and some pesticides (HSDB, 1995). In 1996, the latest year tabulated, the statewide mean outdoor monitored concentration of carbon tetrachloride was approximately 0.08 ppb (CARB, 1999a). The annual statewide industrial emissions from facilities reporting under the Air Toxics Hot Spots Act in California based on the most recent inventory were estimated to be 8781 pounds of carbon tetrachloride (CARB, 2000).

IV.   Effects of Human Exposure

Kazantzis *et al.* (1960) evaluated 17 employees of a quartz processing factory who were occupationally exposed to 45-100 ppm (284-630 mg/m^3) carbon tetrachloride (CCl$_4$) vapor. Fifteen of the 17 workers complained of symptoms including nausea, anorexia, vomiting, flatulence, epigastric discomfort or distention, depressive symptoms, headache or giddiness for up to 4 months prior to the evaluation. A week after CCl$_4$ concentrations were reduced to 0-9 ppm with control measures, workers were symptom-free.

V.   Effects of Animal Exposure

Adams *et al.* (1952) chronically exposed albino Wistar rats, guinea pigs, albino rabbits and rhesus monkeys to 0, 5, 10, 25, 50, 100, 200 and 400 ppm CCl$_4$ for varying duration. For each exposure group, two control groups were devised (unexposed and air-exposed controls) consisting of animals similar in age, sex, weight and number. The 2 control groups responded similarly to the experimental protocol.

In the 100, 200 and 400 ppm exposure groups (Adams *et al.*, 1952), mortality was excessive with moderate to severe liver cirrhosis and other various pathological changes in all the species tested. Fifteen male and 15 female rats were exposed to 50 ppm CCl$_4$ 134 times for 187 days. They experienced decreased body weight gain and liver weight increase as well as moderate fatty degeneration and slight to moderate liver cirrhosis. Females showed kidney weight increase and four rats showed slight to moderate swelling of the kidney tubular epithelium. Guinea pigs (8 males and 8 females; 143 exposures in 200 days) showed depressed growth in the first two weeks, enlarged livers, moderate fatty degeneration and liver cirrhosis, and increased levels of liver total lipids, neutral fat, esterified cholesterol and plasma prothrombin clotting time.

The rabbit group of 2 males and 2 females, which underwent 155 exposures to 50 ppm in 216 days, showed slightly depressed growth and increased kidney weights, prolonged plasma prothrombin clotting time, and moderate fatty degeneration and cirrhosis of the liver.

No change was seen in the group of 2 male monkeys exposed 198 times to 50 ppm in 277 days (Adams *et al.*, 1952). One monkey experienced depressed weight gain compared to the other monkey and the controls, but no other adverse effects were seen with respect to organ weights, tissue examination, total liver lipid, blood urea nitrogen, blood non-protein nitrogen, serum phosphatase, plasma prothrombin clotting time, phospholipid, neutral fat, and free esterified cholesterol.

At 25 ppm CCl$_4$, 15 male and 15 female rats were exposed 137 times for 191 days. Early growth depression in males was observed, although final body weights did not significantly differ from the controls. Significant liver weight increase and slight to moderate fatty degeneration occurred. Liver lipid content was nearly twice the level of the controls and esterified cholesterol was five times that of the controls. For this exposure, phospholipid and neutral fat were not measured. Five male guinea pigs were exposed 133 times over 185 days and 5 female guinea pigs were exposed 93 times over 126 days. Symptoms included growth depression, liver weight

increase, increased plasma prothrombin clotting time, slight to moderate fatty degeneration, twice the level of the control total liver lipid, and five times the control level of esterified cholesterol. After 178 exposures to 25 ppm over 248 days, rabbits (2 per sex) showed increased liver weights and slight to moderate liver cirrhosis and fatty degeneration.

Twenty male and 20 female rats were exposed 136 times over a period of 192 days to 10 ppm CCl_4. These rats exhibited increase in liver weight, slight to moderate fatty degeneration and total lipid, neutral fat and esterified cholesterol levels that were twice the control levels. Guinea pigs (8 male and 8 female), which were exposed 139 times over 197 days, experienced liver weight increase, slight to moderate fatty degeneration without cirrhosis, and increased levels of total lipid, neutral fat, and esterified cholesterol. In an additional group of 18 male rats exposed 13 times to 10 ppm, slight fatty degeneration was seen as early as 17 days. Two male and two female rabbits tolerated the same regimen as the guinea pigs and showed no symptoms as a result of the exposure. Sixteen additional guinea pigs developed hepatic changes after 12 exposures in 16 days.

Twenty-five male and 23 female rats, exposed 145 times over 205 days to 5 ppm CCl_4, had no adverse effects. Nine male and nine female guinea pigs exposed 143 times over 203 days showed a statistically significant increase in the liver weights (females only), but only slightly higher liver lipid content. No additional histopathological effects were seen at this level of exposure.

In a more recent study, Prendergast *et al.* (1967) exposed 15 Long-Evans or Sprague-Dawley rats, 15 guinea pigs, 3 rabbits, 2 dogs, and 3 monkeys 30 times to a concentration of 515 $\pm$39 mg/m^3 (81.7 ppm) carbon tetrachloride (CCl_4) 8 hours a day, 5 days a week, for 6 weeks. (This intermittent exposure is equivalent to a continuous exposure to 123 mg/m^3.) Additionally, two 90 day continuous exposure studies were conducted. One study exposed 15 rats, 15 guinea pigs, 2 rabbits, 2 dogs and 3 monkeys to 61$\pm$5.2 mg/m^3 CCl_4 and the other exposed 15 rats, 3 rabbits, 2 dogs and 3 monkeys continuously to 6.1$\pm$0.3 mg/m^3 CCl_4 in inhalation chambers. Control groups consisted of 304 rats, 314 guinea pigs, 34 dogs, 48 rabbits and 57 monkeys. All the animals' weights were recorded prior to the study, at monthly intervals throughout the study, and at the conclusion of the study.

During the 6 week study, one monkey died following the 7th exposure, and 3 guinea pigs died following the 20th, 22nd, and 30th exposures, respectively. Monkeys, guinea pigs, dogs and rabbits all exhibited weight loss. A high percentage of mottled livers was seen in all species except dogs. Histopathologic examination of the lungs and livers showed morphological changes in all the animals exposed to CCl_4 (most prominently the guinea pigs). The guinea pigs were the most sensitive species displaying discolored lungs, fatty livers, bile duct proliferation, fibrosis, focal inflammatory cell infiltration, hepatic cell degeneration and regeneration, early portal cirrhosis, and alteration of lobular structure. Hepatic lipid content in the guinea pigs was 35.4$\pm$10.7% compared to the control value of 11.0$\pm$3.6%. Alterations of liver lipid content were also observed, to a lesser extent, in the other four species; the most severe alteration occurred in the rats, less severe alteration in rabbits and dogs, and the least severe in the monkeys.

During the 61 mg/m^3 (9.7 ppm) CCl$_4$ continuous exposure study, 3 guinea pigs died (one each after 47, 63, and 71 days). All the monkeys were emaciated and experienced hair loss. Depressed body weight increases were seen in all exposed animals compared to the controls. Autopsies showed enlarged and/or discolored livers in a high percentage (not given) of monkeys, guinea pigs, rabbits, and rats. Rats and guinea pigs showed hepatic fatty acid changes, and a moderate reduction in succinic dehydrogenase activity was also evident in guinea pigs. Varying but lesser degrees of these changes were also seen in the other species tested.

The low concentration of 6.1 mg/m^3 (1 ppm) CCl$_4$ was attained by diluting the CCl$_4$ to 10% of the above concentration with *n*-octane, resulting in a solution of 6.1 mg/m^3 CCl$_4$ in 61 mg/m^3 of *n*-octane (Prendergast *et al.,* 1967)). The level of *n*-octane used was shown to be nontoxic by an *n*-octane control, which yielded no effects. (The current TLV for n-octane is 1400 mg/m^3 (300 ppm) (ACGIH, 1992).) No animals died during this study, and no signs of toxicity were noted. All exposed animals except the rats showed reduced weight gain when compared to the controls, and all species exhibited nonspecific inflammatory lung changes. Guinea pig liver lipid contents and serum urea nitrogen concentrations were similar to the control values. In several animals there were some nonspecific inflammatory changes in the liver, kidney and heart, but the authors did not attribute these to the chemical exposure. There was no other observed hematologic or histopathologic toxicity at this level.

Shimizu *et al.* (1973) exposed groups of 4 female Sprague-Dawley rats to 10, 50 and 100 ppm of CCl$_4$ vapor for 3 hours a day, 6 days a week for up to 6-8 weeks. The rats were terminated two days after the last inhalation. Accumulation of CCl$_4$ occurred in the adipose tissue and was measured after 1 and 3 weeks of exposure. For the 10 ppm group, accumulation was gradual, reaching a level of 1/3 the amount found in the 50 ppm group after 6 weeks. A slight increase of triglycerides in the liver (6.2-6.4 mg/g) was observed in the 10 ppm group, but no control group was used for comparison.

The intermittent exposure caused a more pronounced and higher number of change indices to occur (34 as opposed to the 17 change indices of the monotonous regimen), indicating a greater intensity of liver damage. Changes included a significant decrease in hippuric acid synthesis, presence of mitochondrial enzymes (glutamate dehydrogenase and ornithine carbonyl transferase) in the blood (indicating severe damage to hepatocytes), significant increase in cytoplasmic enzyme activity, and a decrease in the level of cytochrome P-450 in liver tissue. The effects seen in the monotonous group were the same variety as those in the intermittent group, but were less intense. The content of CCl$_4$ in the blood was similar for both the intermittent and monotonous exposure groups. Another test was performed over a period of 27 days varying the regimen, and therefore the concentration, of intermittent exposure while keeping the TWA level of CCl$_4$ stable. Increasing the concentration threefold or fivefold with five 10 minute peaks did not potentiate the toxic effects. Varying the regimen tenfold to five 5-minute peaks (peak exposure 402 mg/m^3 (63.8 ppm)) with a time weighted average exposure of 6.5 ppm (41$\pm$1 mg/m^3) did, however, result in more severe liver damage.

Sakata *et al.* (1987) exposed 10-15 male Sprague-Dawley rats to <10 ppm CCl$_4$ vapor for 15 minutes a day, twice a week for 8 weeks. All the rats had chronic liver damage involving

nodular liver surfaces and extensive fibrosis. Researchers also found similar results in rats after 8 weeks of subcutaneous injections of 0.1 mL of 50% CCl$_4$ solution in olive oil twice a week.

Ideura *et al.* (1993) exposed male Wistar rats to CCl$_4$ vapor for 7 minutes, 3 times a week for 6-10 weeks (concentration unspecified). Six experimental groups of 4-5 rats were used, two of which were exposed for 10 weeks, another two for 6 weeks, and two unexposed control groups. Following the last exposures, rats were injected with varying amounts of endotoxin (1.0 mL lipopolysaccharide (LPS)). The rats were sacrificed 24 hours after the injection and processed for histological examination. Examination of the rats' left kidneys and livers revealed liver cirrhosis with destruction of normal structure and massive ascites retention after 10 weeks of exposure as compared to the controls. Those exposed for 6 weeks exhibited an increase in fibrous tissue. The control groups displayed normal liver structure. Researchers found that rats previously resistant to endotoxin became susceptible following CCl$_4$ exposure, which was manifested as induced acute renal tubular necrosis in cirrhotic rats.

Yoshimura *et al.* (1993) performed a similar experiment to that of Ideura *et al.* (1992) by exposing male Wistar rats for 6 (5 rats) and 10 weeks (5 rats) to 99% CCl$_4$ vapor for 3 minutes a day. A control group of 5 rats was given phenobarbitone for 10 weeks. After 24 hours following the final exposure, rats were injected with endotoxin. Six weeks of CCl$_4$ exposure caused liver fibrosis with bridging fibrosis, while 10 weeks of exposure to CCl$_4$ caused liver cirrhosis and destruction of the normal liver architecture.

Pregnant rats were exposed to 0, 300, or 1000 ppm (0, 1938, or 6460 mg/m^3) carbon tetrachloride for 7 hours/day on days 6-15 of gestation (Schwetz *et al.*, 1974). Significant fetal growth retardation, measured by decreased crown-rump length and body weight, was observed in the offspring of the exposed groups (n = 22 litters) compared with controls (n = 43 litters). Subcutaneous edema was observed in the 300 ppm group but not in the 1000 ppm group. Sternebral anomalies were observed in the 1000 ppm group.

Effects of Chronic CCl$_4$ Exposure (Adams *et al.*, 1952)

Species	Concentration (ppm)	Group size	Endpoint	Exposure scenario (days exposed/ experiment length)
Rats (male)	50 ppm	15	liver damage: fatty degeneration and cirrhosis; growth depression	134/187
Rats (female)	50 ppm	15	same effects as males with the addition of increased kidney weight	134/187
Guinea pigs	50 ppm	16	liver damage: fatty degeneration and cirrhosis; growth depression	143/200
Rabbits	50 ppm	4	enlarged kidney; liver damage: fatty degeneration and cirrhosis; growth depression	155/216
Monkeys	50 ppm	2	one experienced growth depression	198/277
Rats	25 ppm	30	liver damage; early growth depression	137/191
Guinea pigs (male)	25 ppm	5	liver damage: fatty degeneration; growth depression	133/185
Guinea pigs (female)	25 ppm	5	liver damage: fatty degeneration; growth depression	93/126
Rabbits	25 ppm	4	liver damage: fatty degeneration; and cirrhosis	178/248
Rats	10 ppm	40	liver damage: fatty degeneration	136/192
Guinea pigs	10 ppm	16	liver damage: fatty degeneration	139/197
Rats	5 ppm	48	no adverse effects	145/205
Guinea pigs (male)	5 ppm	9	no adverse effects	143/203
Guinea pigs (female)	5 ppm	9	liver damage	143/203

Data from Guinea Pigs and Rats Exposed to 5 ppm CCl_4 for 7 Months (Adams *et al.*, 1952)

←----- g organ weight/g body weight---→

Group	n	BW (g)	Lung	Heart	Liver	Kidneys
Rats, male						
Unexposed controls	11	336	0.65	0.32	2.38	0.65
Air-exposed controls	16	322	0.62	0.31	2.25	0.66
5 ppm CCl_4	13	336	0.62	0.31	2.23	0.65
Rats, female						
Unexposed controls	14	204	0.86	0.38	2.41	0.73
Air-exposed controls	17	209	0.76	0.37	2.76	0.76
5 ppm CCl_4	18	214	0.81	0.38	2.58	0.73
Guinea pigs, male						
Air-exposed controls	7	695	0.79	0.27	3.07	0.63
5 ppm CCl_4	8	669	0.82	0.27	3.14	0.65
Guinea pigs, female						
Air-exposed controls	9	611	0.81	0.27	2.58	0.59
5 ppm CCl_4	6	636	0.78	0.26	2.82*	0.57

* p =0.004

VI.   Derivation of Chronic Reference Exposure Level (REL)

Study	Adams *et al.* (1952)
Study population	9 male and 9 female guinea pigs
Exposure method	Discontinuous whole-body inhalation
Critical effects	Increase in liver weight and liver lipid content in females
LOAEL	5 ppm
NOAEL	Not observed
Exposure continuity	7 hours/day, 5 days/week
Average experimental exposure	1.0 ppm
Human equivalent concentration	1.7 ppm (gas with systemic effects, based on RGDR = 1.7 for lambda (a) : lambda (h) (Gargas *et al.* 1989))
Exposure duration	143 exposures over 203 days (7.3 months)
LOAEL uncertainty factor	3 (mild effect; only in one sex of one species)
Subchronic uncertainty factor	3 (7.3 mo/6 yr guinea pig life-span = 10.1%)
Interspecies uncertainty factor	3
Intraspecies uncertainty factor	10
Cumulative uncertainty factor	300
Inhalation reference exposure level	0.006 ppm (6 ppb; 40 μg/m^3; 0.04 mg/m^3)

Of the 2 adequate chronic inhalation studies available on CCl_4, the Adams *et al.* (1952) study was chosen over the Prendergast *et al.* (1967) study as the key reference for the carbon tetrachloride chronic REL. The Adams *et al.* (1952) experiment was conducted over a longer

duration. In addition, the Adams study contained more specific endpoints of liver damage that were consistent with the mechanism of carbon tetrachloride toxicity. Both studies resulted in hepatic effects with exposed rats appearing less sensitive than the affected monkeys or guinea pigs.

For comparison, conversion of the oral U.S. EPA RfD value of 0.7 µg/kg/day to an equivalent inhalation value by route-to-route extrapolation yields an inhalation REL estimate of 2.5 µg/m^3. As another comparison, if the 6.1 mg/m^3 continuous exposure in Prendergast *et al*. (1967) is a NOAEL (for rats), the resulting REL estimate would be 60 µg/m^3. If the 6.1 mg/m^3 continuous exposure is a mild LOAEL, the resulting REL estimate would be 20 µg/m^3.

VII.   Data Strengths and Limitations for Development of the REL

The major strengths of the REL for carbon tetrachloride are the chronic exposure study used and the target tissue affected. The major uncertainties are the lack of human data, the lack of a NOAEL observation, the small sample sizes used, and the lack of comprehensive multiple dose studies.

VIII.   References

Adams E, Spencer H, Rowe V, McCollister D, and Irish D. 1952. Vapor toxicity of carbon tetrachloride determined by experiments on laboratory animals. Arch. Indust. Hyg. Occup. Med. 6(1):50-66.

ACGIH. 1992. American Conference of Governmental and Industrial Hygienists. Documentation of the Threshold Limit Values and Biological Exposure Indices. 6th ed. Vol. I. Cincinnati, OH: ACGIH.

CARB. 1999. California Air Resources Board. Toxics Air Quality Data. Substance Chooser. Carbon tetrachloride. Available online at http://www.arb.ca.gov/aqd/toxics.htm

CARB. 2000. California Air Resources Board. California Emissions Inventory Development and Reporting System. (CEIDARS). Data from Data Base Year 1998. February 12, 2000.

CRC. 1994. CRC Handbook of Chemistry and Physics, 75th edition. Lide DR, ed. Boca Raton, FL: CRC Press Inc.

DeShon ND. 1979. Carbon Tetrachloride. Kirk-Othmer Encyclopedia of Chemical Technology, 3rd ed. Grayson M, and Eckroth D, eds. New York: John Wiley and Sons, Inc. 5:704-714. [as cited by Santodonato J. 1985. Monograph on human exposure to chemicals in the workplace: Carbon tetrachloride; PB86-143377; SRC-TR-84-1123.]

Gargas ML, Burgess RJ, Voisard DE, Cason GH, and Andersen ME. 1989. Partition coefficients of low-molecular-weight volatile chemicals in various liquids and tissues. Toxicol. Appl. Pharmacol. 98(1):87-99.

HSDB. 1995. Hazardous Substances Data Bank. National Library of Medicine, Bethesda, MD (CD-ROM version). Denver, CO: Micromedex, Inc. (edition expires 7/31/95).

Ideura T, Yoshimura A, Shirai M, Taira T, and Koshikawa S. 1993. Endotoxin induced acute tubular necrosis in cirrhotic rats. Scand. J. Urol. Nephrol. 27:433-439.

Kazantzis G, Bomford RR, and Oxon DM. 1960. Dyspepsia due to inhalation of carbon tetrachloride vapour. Lancet 360-362.

Prendergrast J, Jones R, Jenkins L, and Siegel J. 1967. Effects on experimental animals of long-term inhalation of trichloroethylene, carbon tetrachloride, 1,1,1-trichloroethane, dichlorodifluoromethane, and 1,1-dichloroethylene. Toxicol. Appl Pharmacol 10:270-289.

Sakata T, Watanabe N, Hobara N, and Nagashima H. 1987. Chronic liver injury in rats by carbon tetrachloride inhalation. Bull. Environ. Contam. Toxicol. 38:959-961.

Santodonato J. 1985. Monograph on human exposure to chemicals in the workplace: Carbon tetrachloride; PB86-143377; SRC-TR-84-1123. NTIS.

Schwetz BA, Leong BK, and Gehring PJ. 1974. Embryo - and fetotoxicity of inhaled carbon tetrachloride, 1,1-dichloroethane and methyl ethyl ketone in rats. Toxicol. Appl. Pharmacol. 28:452-464.

Shimizu Y, Nagase C, and Kawai K. 1973. Accumulation and toxicity of carbon tetrachloride after repeated inhalation in rats. Ind. Health 11:48-54.

Yoshimura A, Ideura T, Shirai M, Tiara T, Iwasaki S, Kitaoka T, and Koshikawa S. 1993. The distribution of ^{3}H-labeled endotoxin in the kidney of liver cirrhotic rats. Scand. J. Urol. Nephrol. 27:295-299.